USACEHR TECHNICAL REPORT 1002

An Evaluation of the Eclox Chemiluminescence Test, Hach Pesticide/Nerve Agent Test Strips, and Agri-Screen Test Tickets

David E. Trader
William H. van der Schalie, Ph.D.

Approved for public release; distribution is unlimited

30 June 2010

United States Army Center for Environmental Health Research
Fort Detrick, MD 21702-5010

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
30-06-2010	Research	28 August 2010 - 10 June 2010

4. TITLE AND SUBTITLE

An Evaluation of the Eclox Chemiluminescence Test, Hach Pesticide/Nerve Agent Test Strips, and Agri-Screen Test Tickets

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

622787

6. AUTHOR(S)

David E. Trader and William H. van der Schalie

5d. PROJECT NUMBER

FH2

5e. TASK NUMBER

3D

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Center for Environmental Health Research
568 Doughten Drive
Fort Detrick, MD 21702-5010

8. PERFORMING ORGANIZATION REPORT NUMBER

TR 1002

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
504 Scott Street
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for Public Release; Unlimited Distribution.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The United States Army Center for Environmental Health Research (USACEHR) has developed an Environmental Sentinel Biomonitor (ESB) system to test Army drinking water supplies for the presence of toxic industrial chemicals (TICs). One of the technologies considered for inclusion in the ESB system was the Eclox Chemiluminescence Test, which, along with the Pesticide/Nerve Agent (P/NA) Test Strip is available as part of the Hach Eclox Rapid Response Water Toxicity Kit. The Eclox test and P/NA Test Strips are simple, rapid tests with sturdy packaging and materials, and the reagents used are stable for up to one year. However, the Eclox and P/NA Test Strip combination responded to only 2 of 21 chemicals in the desired sensitivity range between the military exposure guideline and human lethal concentrations for each chemical.

15. SUBJECT TERMS

biosensor, toxicity sensor, aquatic toxicity testing, pesticide, organophosphate, carbamate, chemiluminescence, horseradish peroxidase, HRP, acetylcholinesterase, Eclox, Neogen, Agri-Screen, Hach

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Carol O'Brien
U	U	U	SAR	48	19b. TELEPHONE NUMBER *(Include area code)* 301-619-7685

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

Table of Contents

List of Figures and Tables

Figures

Tables

1. Introduction

1.1 The Environmental Sentinel Biomonitor System

The United States Army Center for Environmental Health Research (USACEHR) has developed an Environmental Sentinel Biomonitor (ESB) system to test Army drinking water supplies for the presence of toxic industrial chemicals (TICs). For the first increment (Increment 1) of the ESB system, two sensor components were chosen: Electrical Cell-substrate Impedance Sensing (ECIS) and the Abraxis Organophosphate/Carbamate (OP/C) Screen pesticide assay. Acceptable concentration ranges for toxicity detection in the ESB system (van der Schalie et al., 2006) were determined to be above the Military Exposure Guideline (MEG) concentration (based on consumption of 15 liters (L) per day of water for 7 – 14 days; (USACHPPM, 2004) and below the estimated human lethal concentration (HLC) (based on the consumption of 15 L of water per day for a 70 kilogram (kg) person; TERA, 2006). Together, the ECIS and OP/C assays responded to 14 out of 20 of the TICs within the MEG-HLC range.

Several technologies besides the ECIS and OP/C assays were considered during the downselection process for Increment 1 by an integrated product team (IPT). The IPT was particularly interested in the Eclox Chemiluminescence Test because of its rugged design and apparent suitability for field use. However, it was not selected for further testing because it responded to only 1 of 12 chemicals below the HLC (van der Schalie et al., 2006). Other literature sources indicate that another of the 12 chemicals, cyanide, may be detected well below the MEG (States et al., 2003).

Currently, the Eclox Chemiluminescence Test is available as part of the Hach Eclox Rapid Response Water Toxicity Kit; another component of the kit is the Hach Pesticide/Nerve Agent (P/NA) Test strip. It is believed that the Hach P/NA Test strip is similar to the Neogen® Agri-Screen Test, which was previously subjected to a preliminary range-find round of testing (see Table 1-1, Buehler, 2008). Buehler concluded that the Agri-Screen Test detected 1 pesticide (methyl parathion) of the 5 organophosphate and carbamate pesticides tested in the MEG-HLC range. Further, the Hach P/NA Test strip provided with the Eclox Rapid Response Water Test Kit does not include an oxidation or "activator" step, as the described in the Agri-Screen procedure (Appendix A). The oxidation step is used to improve detection of the thio form (S=P) of OP pesticides (Mr. Kevin Morrissey, personal communication).

Although the Abraxis OP/C Screen has been shown to be more sensitive to the set of 5 organophosphate/carbamate chemicals (Trader et al., 2009) than the Agri-Screen Test, the simplicity of this kit and the P/NA Test strip make them very attractive for field use. In fact, while both the P/NA Test strip and the Eclox Chemiluminescence Test appear to have limited sensitivity to toxicants, they have been widely used. In August 2009, Federal Emergency Management Agency listed the Eclox Rapid Response Water Test Kit as the only broad-band biosensor in the "Responder Knowledge Base" product listing.

Table 1-1: Evaluation of Commercially-Available Test Kits Using Selected Pesticides

Test Chemicals	MEG[a]	Abraxis	Agentase	Enviro-logic	Neogen ®	Oritest	Strategic Diagnostic	HLC[b]
Aldicarb	0.005[c]	**0.05**	>0.17	<0.005	>0.17	>0.17	0.5	0.17
Fenamiphos	0.004	**0.56**	>0.56	<0.0004	>0.56	>0.56	>0.56	0.56
Methamidophos	0.002	**14-140**	>1.4	<0.002	>1.4	>1.4	>1.4	1.4
Methyl parathion	0.15	**0.15**	>33.1	<0.015	33.1	>33.1	>33.1	33.6
Oxamyl	0.1	**0.1**	>0.63	<0.001	>0.63	>0.63	>0.63	0.63
Water Blank #1		**ND**[d]	ND	Yes	ND	ND	ND	
Water Blank #2		**ND**	ND	ND	ND	ND	ND	

	Chemical detected in MEG-HLC range
	Chemical detected below MEG value
	Chemical detected above HLC value

[a] **MEG** – 7 to 14 day Military Exposure Guidelines (15 liter [L]/day), when available; fenamiphos MEG estimated from terbufos (Richards, personal communication)
[b] **HLC –** Human Lethal Concentration (70 kilogram [kg] person, 15 L/day)
[c] All concentrations reported as milligram/liter
[d] **ND –** No Detection
Testing completed by Battelle (Buehler, 2008)

The Department of Homeland Security has awarded grants for purchasing the Eclox (Severn Trent Services, Inc., 2006 and Henderson, 2005). The British Ministry of Defence has been using the Eclox chemiluminescence test since 2001 (Jane's, 2002). As a result of widespread interest in use of the Eclox and its potential for field use by the Army, further toxicant testing was desired so that the Eclox test along with the pesticide test strips, could be included as part of the increment 2 ESB system downselection process.

The increment 2 ESB system is intended for use in conjunction with Army field water production equipment at levels II and III of Army preventative medicine support in the theaters of operation. This requires improvements to the first increment in two major areas: responsiveness to chemicals and reductions in the size and logistical requirements of the system. The Eclox and associated pesticide test strips have the potential to offer:

- Improved storage (4 months at +40 degrees Celsius(°C) and 1 year in refrigeration);
- Improved ruggedness (sturdy design with no pumps, hoses, and few moving parts);
- Improved time-to-results (no pre-exposure, test time reduced from 60 minutes to 4 minutes); and
- Dramatic reduction in size of the ESB platform.

The goal of this project is to evaluate the responses of the Eclox and associated pesticide test strips to the 21 ESB test chemicals and 5 interference chemicals, as outlined in previous ESB testing (Trader et al., 2009). In addition, toxicant response information can

be harmonized with previous in-house sensor testing (Trader et al., 2009 and Widder et al., 2007)

1.2 The Eclox Chemiluminescence Test

The Eclox Chemiluminescence Test utilizes horseradish peroxidase to catalyze luminol in the presence of both an oxidant (sodium perborate) and enhancer (luminol and para-iodophenol). This reaction produces a flash of light which is read by a luminometer. The luminometer measures the sample in light intensity units (arbitrary). Chemicals that interfere with the reaction cause a decreased light intensity output. This output is compared to a water blank, thereby generating a percent (%) inhibition.

1.3 Pesticide/Nerve Agent Test Strips

Minimal technical data is available for the Hach P/NA Test strip, but the technology appears to be similar to the Agri-Screen Test, where membrane-bound cholinesterase is exposed to a chromogen and will result in a negative sample turning blue color. Chemicals will interfere with the cholinesterase, which is required to complete the color change to blue, and the result is a white disc (indicating a positive for a pesticide/nerve agent). Simple detect/non-detect information is generated based on observation of a color change to each strip.

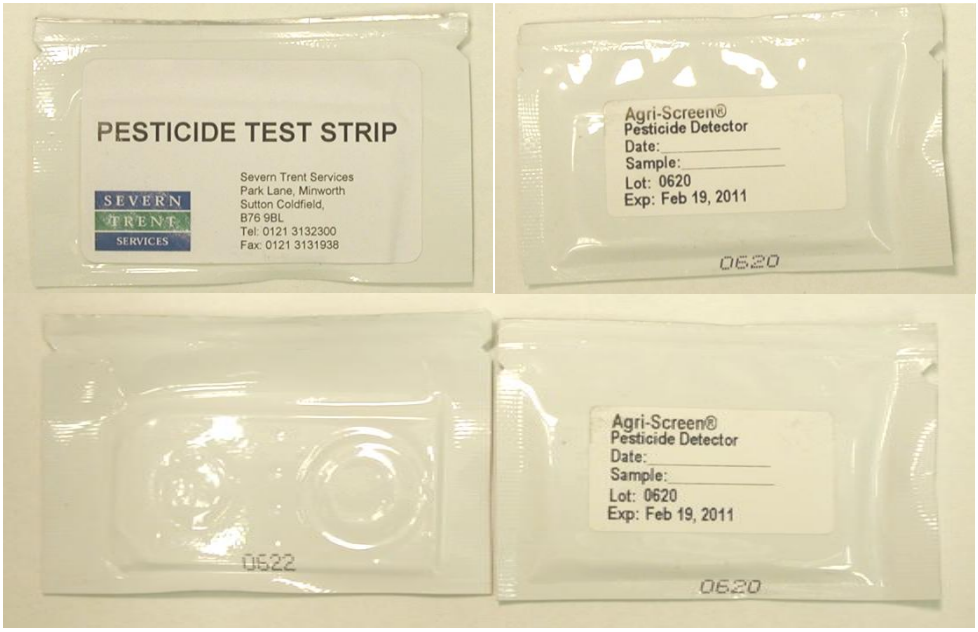

Figure 1-1. Photographs of the Hach Pesticide/Nerve Agent Test strip (left) and the AgriScreen Test Ticket (right); notice the similarity in their packaging. Note the stock numbers are similar on the Hach P/NA Test strip (lower left) and the Agri-Screen ticket (lower right). Because of these similarities and their similar responses to the pesticides, we felt these were the same product (see text for details).

2. Materials and Methods

2.1. Eclox Chemiluminescence Test Method Summary

This section describes manufacturer's instructions for using the Eclox Chemiluminescence Test.

2.1.1 Test Method Modifications

All procedural steps for the Eclox test are found in Section 3, 4, and 11 of the user's manual (Eclox Water Test Kit Manual, 2006). The test procedure (Appendix B) described in user's manual was followed with the following changes. To minimize test variability, the samples were pulse-vortexed after all three reagents were added instead of triturated (mixed using a pipette tip) prior to insertion into the luminometer. A blank sample was tested before any toxicant samples each day; percent inhibition had to be between -9% and 9% or new reagents were made based on Texas Engineering Extension Services (TEEX) data and recommendations (McLeroy, personal communication). A positive control (phenol at 1 milligram [mg]/L) was tested before any toxicant samples each day and had to have a percent inhibition between 40% and 60%. If the positive control did not fall in this range, a second replicate was completed, the reagents were vortexed, and procedures were examined. A stock solution of phenol at 5 mg/L was used as the positive control, and was diluted using 200 microliter (µL) of phenol, 800 µL of Millipore water, yielding 1 ml of a 1 mg/L positive control concentration (as per TEEX procedure). Reagent bottles were vortexed prior to use each day for uniform mixing. Reagents were delivered using lab-calibrated pipettors instead of the manufacturer-provided pipettors.

2.1.2 Eclox Preparation and Environmental Conditions

Information regarding the calibration of the luminometer is found in section 4 of the user's manual (Eclox Water Test Kit Manual, 2006.) Information regarding the preparation of the reagents is found in section 3 of the user's manual (Eclox Water Test Kit Manual, 2006.) On the day of each test, reagents 1, 2 and 3 were either prepared or removed from refrigeration and placed at room temperature (20-25°C) for at least 30 minutes prior to testing (room temperature recordings were within the range of 20-24°C). The luminometer has a series of current, light and other internal checks to ensure proper operation.

2.1.3 Eclox Endpoint and Minimum Detection Limit

The % inhibition was the Eclox endpoint, and an inhibition of 25% or greater was considered a "detect." Inhibition less than 25% or enhancement (negative % inhibition) was considered a non-detect. A statistical analysis of negative blank samples (Millipore water) was completed to determine the likelihood of false positive responses. Based on data from 30 blank samples, a 20% inhibition threshold gave a false positive rate of less

than 1 in 10,000 (Appendix C). For testing purposes, a more conservative estimate of the threshold was established at 25% inhibition.

Toxicant testing used the statistical approach for the Joint Chemical Biological Radiological Agent Water Monitor (JCBRAWM) program to determine a minimum detection limit (MDL) for each test chemical, defined as a concentration at which there is a probability of detection of 90%, with 80% confidence (Hogan et al., 2007). Based on binomial probabilities, this requires that a minimum of 16 of 16 samples be detected (inhibition $\geq$ 25%) with no false negatives (inhibition < 25%), or that at least 29 of 30 samples be similarly detected.

2.1.4 Eclox Data Analysis

Raw intensity values are provided by the luminometer and are graphed for the reference and each sample over 4 minutes (min). A % inhibition is automatically generated by the Eclox software within the luminometer based on the following calculations, adapted from ASTM 6592-01, where the result is expressed as % inhibition of the integrated results:
Reference:

$$REF = \sum_{nR=1}^{240} nRi$$

where
nR = an intensity value from the luminometer

Sample:

$$T_{4\,\min} = (1-[(\sum_{nT=1}^{240} nTi) / REF]) \times 100\%.$$

where:
nT = an intensity value from the luminometer

Generally speaking, the % inhibition is calculated by: 1 minus the sum of 240 sample intensity readings (1 per second for 4 minutes) divided by the sum of 240 reference intensity readings, with this quantity multiplied by 100%.

2.1.5 Eclox Materials

The materials used were:

- Hach Eclox Rapid Response Water Test Kit; Cat #2886800, GSA# GS-07F9314S, Hach Company, Loveland CO.
- Chemiluminescence Reagent Set; Cat#2887500 for 50 or #949004 for 100, Hach Company, Loveland CO.
- Polystyrene 3.5 round bottom cuvettes; Cat #2887400 for 20, #30-0015 for 500 Hach Company, Loveland CO. (available from Starstedt as well, Cat#55.484).
- Pesticide Test Strips; Cat#2887600 for 10, Hach Company, Loveland, CO.

- Agri-Screen Starter Kit; Cat#8901, Neogen ® Corporation, Lansing, MI
- 2 µL – 200 µL pipette tips; 05-403-41, Fisher Scientific, Pittsburgh, PA.
- 50 µL – 1000 µL pipette tips; 05-403-43, Fisher Scientific, Pittsburgh, PA.

2.2. Pesticide Test Strip Method Summary

This section describes the materials and methods for both pesticide strip technologies. Because of the many similarities between the two tests, it is suspected that the Agri-Screen and Hach P/NA Test strips are in fact the same test, although this cannot be conclusively proven.

2.2.1 Hach Pesticide/Nerve Agent Test Method

The Hach P/NA Test strip technology has a very simple test method. The strip is removed from its outer packaging and the foil is folded back on the white disk side (corners tapered). The white disk side of the strip is dipped in the test sample (50 ml in a 100 ml beaker, provided by Neogen ® Corp) for 1 minute. The foil is removed and the pink/purple disk side is pinched with the white disk side for 3 minutes. After the 3 minute incubation, the white disk side is opened and viewed. See Figure 2-1 for sample interpretation.

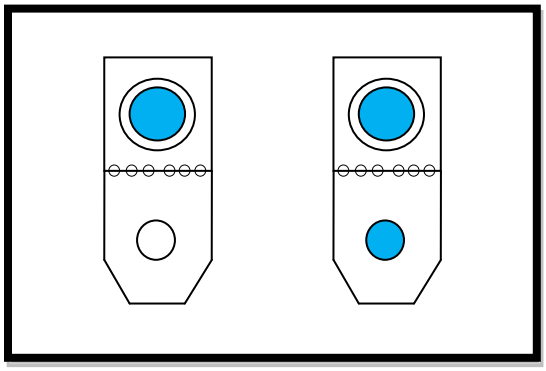

Figure 2-1. Illustration of a positive and negative control result of the strip/ticket technology. A white disk and blue disk are positive (left, detect) and 2 blue disks are negative (right, non-detect).

2.2.2 Neogen ® Agri-Screen Test Method

The only procedural difference between the Hach Test strips and the AgriScreen test tickets is a pre-oxidation step. Before immersing the white disk side of the ticket, a bromine capsule is placed in the 50 ml sample and crushed with a glass rod (Figure 2-2). The bromine is allowed to incubate for 3 minutes prior to immersing the ticket in the sample. The 1 minute white disk dipping and 3 minute pinch is the same. The procedure is similar, as are the visual comparisons (Figures 2-3).

Figure 2-2. Neogen ® Agri-Screen Oxidation Materials. Shows a top view of the bromine capsule container lid, bromine capsules, 100 ml beaker and glass rod provided by Agri-Screen.

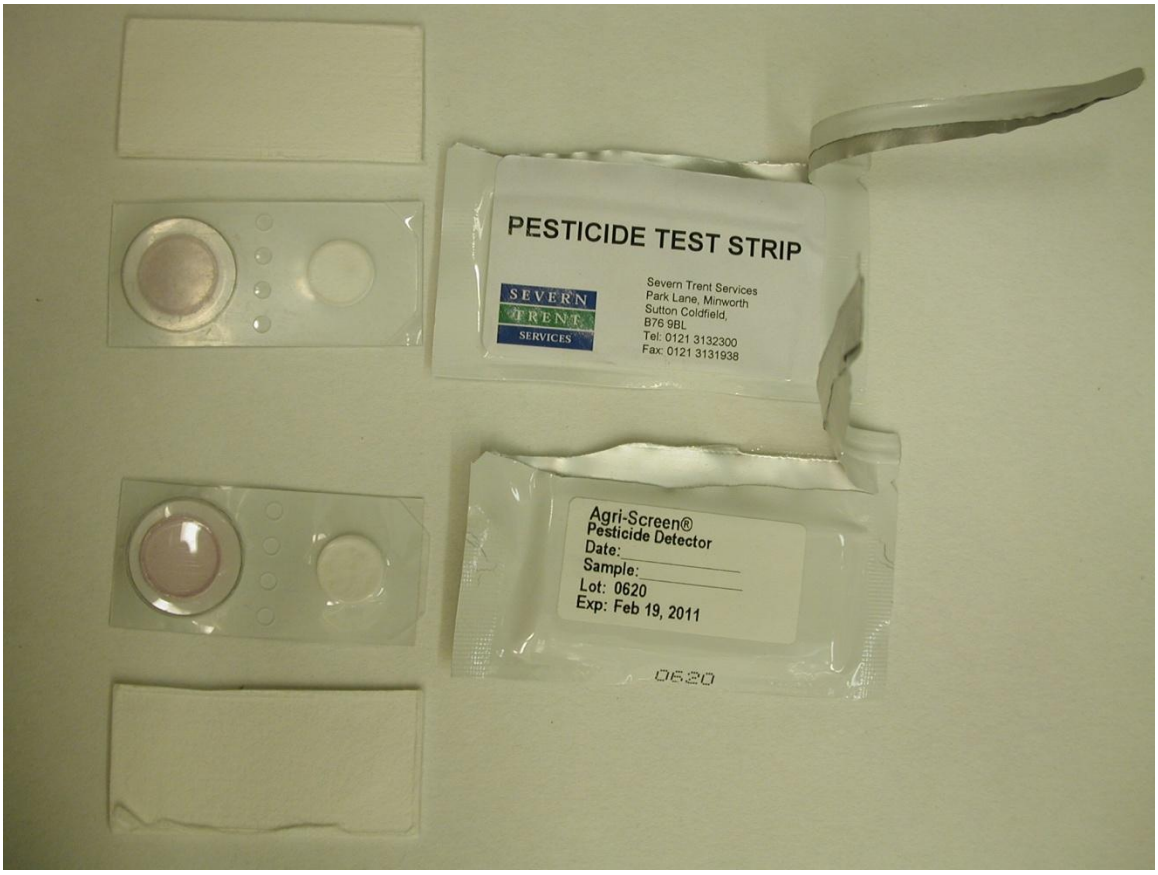

Figure 2-3. Opened Hach Pesticide/Nerve Agent Test strip and Neogen ® Agri-Screen Ticket. The opened, unused test tickets with similar matte packaging from the Eclox Rapid Response Kit (top) and Agri-Screen.

All samples tested on the test strips were photographed for data storage purposes, and were noted as detect/non-detect in supplemental data records.

The Hach P/NA Test strips that accompany the Chemiluminescence test at the time of purchase were approximately $40 per sample (now available at approximately $16 per sample). The complete testing of all chemicals if purchased from Hach Company would have been cost prohibitive. Therefore, because of the lower costs, pesticide test strips for definitive testing were purchased directly from Neogen ® for $7.50 per sample, instead of using the Hach P/NA Test strips.

2.2.3 Pesticide Test Strip Endpoint and Minimum Detection Limit

A simple observation of white or blue was used as the endpoint for both pesticide test strip technologies. The definition of the MDL concentration for each chemical was the same as described in section 2.1.3.

2.3 Toxicant Testing Methods

The study design of the Eclox Chemiluminescence Test and the pesticide test strip technologies differed in toxicant concentrations tested and the number of replicates used.

2.3.1 Experimental Design of Eclox

The test strategy was to first determine if Eclox could detect a chemical at the HLC, the maximum acceptable response concentration as defined by the IPT. If a response was found at the HLC, the MEG was tested to determine whether the response was within the acceptable MEG-HLC range. Further testing was done to establish the detection level as defined in 2.1.3 for those chemicals where responses were found in the MEG-HLC range. Each of the 21 chemicals listed in Appendix D were tested at the HLC with 3 replicates:

- If the chemical had 0-2 detects of 3 replicates at the HLC, the chemical was tested at 10 times the HLC (3 replicates).
- If the chemical had 3 detects of 3 replicates at the HLC, the chemical was tested at the MEG (3 replicates).
    - If the chemical had 0-2 detects of 3 replicates at the MEG, the chemical was tested with 3 concentrations spaced equally about the geometric mean between the MEG and HLC, each with 3 replicates.
    - If the chemical had 3 detects of 3 replicates at the MEG, the chemical was tested at 10 times lower than the MEG (3 replicates).

The Eclox Chemiluminescence Test was also tested with 16 replicates of each interference at the concentrations listed in Table 2-2.

2.3.2 Experimental Design of Pesticide Test Strips

Each of the five organophosphate or carbamate chemicals reported in Trader et al., 2009, (aldicarb, fenamiphos, methamidophos, methyl parathion, and oxamyl) were tested using the Agri-Screen test ticket at the HLC (3 replicates):

- If the chemical had 0-2 detects of 3 replicates at the HLC, the chemical was tested at 10 times the HLC (3 replicates).

  - If the chemical had 0-2 detects of 3 replicates at the HLC, the chemical was tested at the stock concentration (1 replicate)
- If the chemical had 3 detects of 3 replicates at the HLC, the chemical was tested at the MEG (3 replicates).
  - If the chemical had 0-2 detects of 3 replicates at the MEG, the chemical was tested with 3 concentrations spaced equally about the geometric mean between the MEG and HLC; each with 3 replicates.
  - If the chemical had 3 detects of 3 replicates at the MEG, the chemical was tested at 10 times lower than the MEG (3 replicates).

One replicate using an Agri-Screen test ticket at either the detection limit or the stock concentration (whichever was lower) was tested using the oxidation step provided with the Agri-Screen to see if oxidation improved detection capabilities. There were 16 replicates used to provide definitive results for the chemicals that were detected between the MEG and HLC.

Since it is suspected that the Agri-Screen and Hach P/NA Test strips are the same test, comparative testing was conducted to determine if toxicant response sensitivities are similar. Agri-Screen strips (3 replicates) and the Hach P/NA Test strips (3 replicates) were tested using each of the five organophosphate and carbamate (OP/C) chemicals at the HLC. Non-OP/C chemicals were tested using the same method as the OP/C chemicals, except only 1 replicate of the Hach P/NA Test strips was used for comparison to the 3 replicates of Agri-Screen tickets.

The Agri-Screen tickets were tested with 3 replicates of each interference at the concentration listed in Table 2-2, with 1 additional replicate using the oxidation step and 1 replicate using the Hach P/NA Test strips. If at least 1 replicate was considered a detect for an interference, a detection limit with 16 replicates was determined.

2.4 Test Chemicals

The 21 test chemicals and six interferences used in this evaluation originally were chosen for testing the ESB system by the IPT (Kooistra et al., 2007), and were the same as those used to evaluate other candidate ESB toxicity sensors (Widder et al., 2007). These chemicals were chosen to provide a broad range of potential threat chemicals, modes of toxic action, and likely interfering agents in source or product drinking waters. For testing of candidate toxicity sensors for the increment 2 ESB system, ethylene glycol and oxamyl were removed from the chemical test list and sodium azide was added, for a total of 18 test chemicals. For this evaluation, all 21 test chemicals were tested; these are shown with their MEG and HLC values in Table 2-1. Table 2-2 shows the interference chemicals along with the test concentrations recommended by the IPT.

Table 2-1: Test Chemical Military Exposure Guidelines and Human Lethal Concentration Values		
Test Chemicals[a]	**MEG[b] (mg/L)**	**HLC[c] (mg/L)**
Acrylonitrile	0.14	4.2
Aldicarb	0.005	0.17
Ammonia	30	924
Arsenic (sodium arsenite)	0.02	4.5
Azide (sodium azide)[d]	0.12	47
Copper (sulfate)	0.14	103
Cyanide (sodium)	2	14
Ethylene glycol	2.5	3157
Fenamiphos	0.004	0.56
Fluoroacetate (sodium)	0.0009	3.9
Mercury (chloride)	0.001	24.7
Methamidophos	0.002	1.4
Methyl parathion	0.15	33.6
Nicotine	0.13	16.8
Oxamyl	0.1	0.63
Paraquat (dichloride)	0.05	4.6
Pentachlorophenate (sodium)	0.14	71.9
Phenol	3	91.5
Strychnine	0.014	1.3
Thallium (sulfate)	0.003	13.5
Toluene	1	840

[a] More chemical information available in Appendix D

[b] **MEG** – 7 to 14 day Military Exposure Guidelines (15 liter [L]/day), when available, 1 year MEG for copper, fluoroacetate, and strychnine; < 7 day MEG for nicotine; fenamiphos MEG estimated from terbufos (Richards, personal communication)

[c] **HLC –** Human Lethal Concentration (70 kg person, 15 L/day)

[d] Sodium azide added to the chemical list for increment 2 ESB toxicity sensor testing

Table 2-2: Interferences	
Test Chemicals	**Concentration (mg/L)**
Chlorine	10
Chloramines	10
Geosmin	0.0001
Methyl-isoborneol (MIB)	0.0001
Humic / Fulvic Acids (50%/50%)	5 (2.5/2.5)
Hard Water	250

Chemical stock solutions were prepared in American Society of Testing and Materials (ASTM) Type II water, also referred to as Millipore water in this report. Pentachlorophenate (PCP) (sodium) was prepared by titrating pentachlorophenol in 50 millimolar (mM) phosphate buffer with 1 molar hydrochloric acid to pH 7.5. Test chemicals were used either the same day as analyzed (when possible) or within two weeks in 4°C storage. All test chemicals were verified as stable for two weeks under these storage conditions. Volatile chemicals (acrylonitrile and toluene) were stored in zero headspace vials at 4°C. Three test chemicals (ethylene glycol, fenamiphos, and methamidophos) and three interference chemicals (geosmin, humic/fulvic acids, and methyl-isoborneol) stock solutions were tested at nominal concentrations because suitable methods for analysis at the required concentrations were not available. All other stock concentrations of test compounds were analyzed using the analytical methods indicated in Appendix D. The suitable pH range of the Eclox is 6-8, (Lauer, 2005) although the authors indicate that pH 5.4 to 9.5 are acceptable. The recommended pH range for Agri-Screen is 3-8 (Jacob Clark, Neogen ® Corporation, e-mail communication), so stock solutions were titrated with 1N HCl or 1N NaOH until their pH was in the suitable Eclox testing range (pH 6-8).

---

3. Results and Discussion

3.1 Eclox Testing

3.1.1 Negative Controls

Before each definitive test and throughout testing, a Millipore water sample was tested to gather data regarding negative blank samples. These samples were compared to the daily reference sample (also Millipore water) and a percent inhibition was computed based on this comparison (Figure 3-1). The test protocol stated that an inhibition less than -9 % or greater than 9% should be re-run. Four samples out of 60 were outside this range. None of the samples (0 of 60) were above 25%, which was considered the toxicity threshold. Two consecutive samples above 9% were noted (samples 24 and 25, with 14% and 11% inhibition, respectively); these samples were run the last of the day, and represent the last portion of reagent left in the bottle. A fresh set of reagents was prepared for the next day of testing.

3.1.2 Positive Controls

Before each definitive test, and at the end of each testing day, a sample of phenol at 1 mg/L was tested to gather data regarding positive control samples. These samples were compared to the daily reference sample (Millipore water) and a percent inhibition was computed based on this comparison (Figure 3-2). The test protocol stated that an inhibition less than 40% or greater than 60% should be re-run. No samples out of 37 tested were outside this range. All of the samples (37 of 37) were above 25%, which was considered the toxicity threshold. The % inhibition range for the positive controls was 41% to 55%, and both the mean and median inhibition levels were 50%.

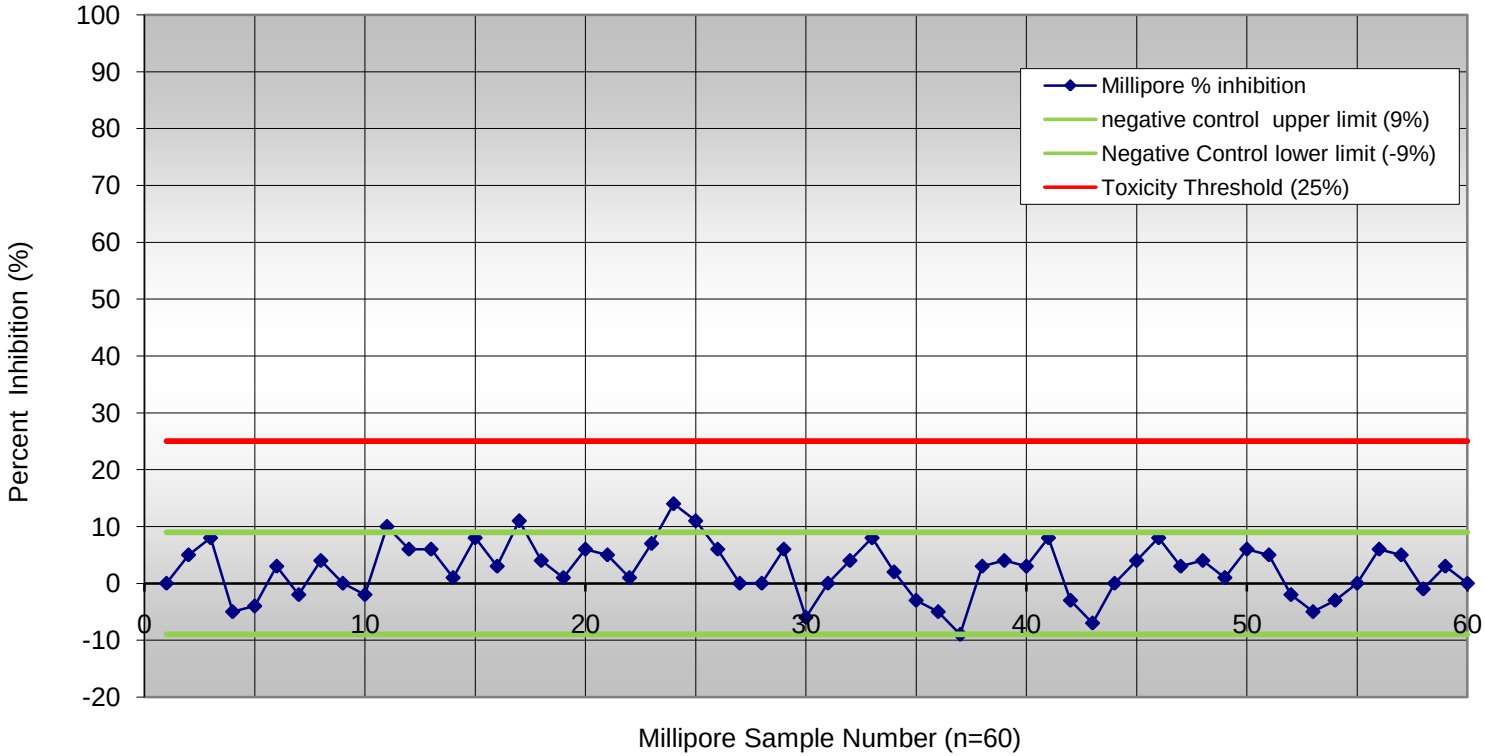

Figure 3-1. Eclox Chemiluminescence Negative Controls. Four of the 60 control samples exceeded 9% inhibition. A red reference line is provided at 25% inhibition, the minimum level established for a positive response.

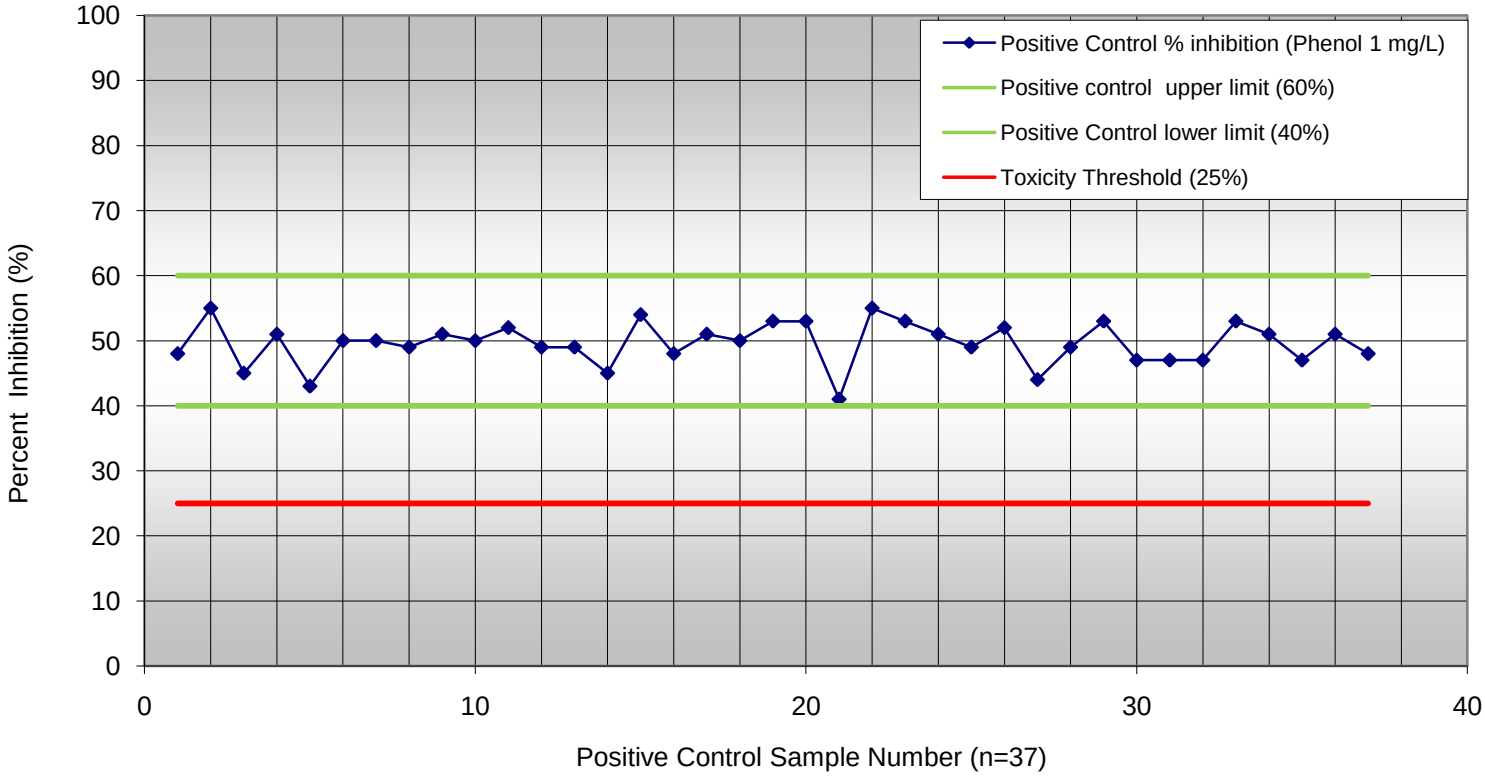

Figure 3-2. Eclox Chemiluminescence Positive Controls. All of the 37 positive control samples were within pre-defined limits. A red reference line is provided at 25% inhibition, the minimum level established for a positive response.

3.1.3 Eclox Chemiluminescence Results

The Eclox Chemiluminescence Test detected two chemicals below the MEG (cyanide and phenol) and one chemical in the MEG-HLC range (mercury). The remaining 18 chemicals were detected either above the HLC or not at all (Table 3-1). Table 3-1 shows the responses for each chemical. Figures 3-3 and 3-4 show the results of 16 replicates of mercury at the HLC and a reference sample (Millipore).

Table 3-1: Eclox Chemiluminescence Test Results

Test Chemicals	MEG[a]	Eclox Chemiluminescence MDL[b]	Eclox Mean % Inhibition	Eclox Response (x of n)	HLC[c]
Acrylonitrile	**0.14**[d]	>4.2	*21*	1 of 3	**4.2**
Aldicarb	**0.005**	>668.8	*15*	0 of 3	**0.17**
Ammonia	**30**	>924	*8*	0 of 3	**924**
Arsenic (sodium arsenite)	**0.02**	46-460	*96*	3 of 3	**4.5**
Azide (sodium)	**0.12**	>47	*0*	0 of 3	**47**
Copper (sulfate)	**0.14**	>103	*18*	0 of 3	**103**
Cyanide (sodium)	**2**	0.2	*40*	30 of 31	**14**
Ethylene glycol	**2.5**	3157	*4*	0 of 3	**3157**
Fenamiphos	**0.004**	0.56 - 204	*63*	3 of 3	**0.56**
Fluoroacetate (sodium)	**0.0009**	>4211	*16*	1 of 3	**3.9**
Mercury	**0.001**	24.7	*44*	19 of 19	**24.7**
Methamidophos	**0.002**	14 - 1008	*59*	3 of 3	**1.4**
Methyl parathion	**0.15**	>33.6	*-1*	0 of 3	**33.6**
Nicotine	**0.13**	16.8-168	*62*	3 of 3	**16.8**
Oxamyl	**0.1**	>6.3	*-2*	0 of 3	**0.63**
Paraquat (dichloride)	**0.05**	> 46	*7*	0 of 3	**4.6**
Pentachlorophenate (sodium)	**0.14**	71.9 - 2154	*30*	3 of 3	**71.9**
Phenol	**3**	1	*50*	29 of 29	**91.5**
Strychnine	**0.014**	>47.9	*12*	0 of 3	**1.3**
Thallium (sulfate)	**0.003**	135-465.5	*34*	3 of 3	**13.5**
Toluene	**30**	>444[e]	*13*	0 of 3	**840**

Legend	Eclox Summary
Chemical detected in MEG-HLC range (≥ 25%)	1
Chemical detected below MEG	2
Chemical detected above HLC	6
Chemical not detected at any concentration tested	12

[a] **MEG** – 7 to 14 day Military Exposure Guidelines (15 L/day), when available, 1 year MEG for copper, fluoroacetate, and strychnine; < 7 day MEG for nicotine; fenamiphos MEG estimated from terbufos (Richards, personal communication)

[b] **MDL** – Minimum Detection Limit where n=16 with 0 false negatives

[c] **HLC –** Human Lethal Concentration (70 kg person, 15 L/day)

[d] All concentrations reported as milligrams/liter,

[e] Toluene stock measured at solubility

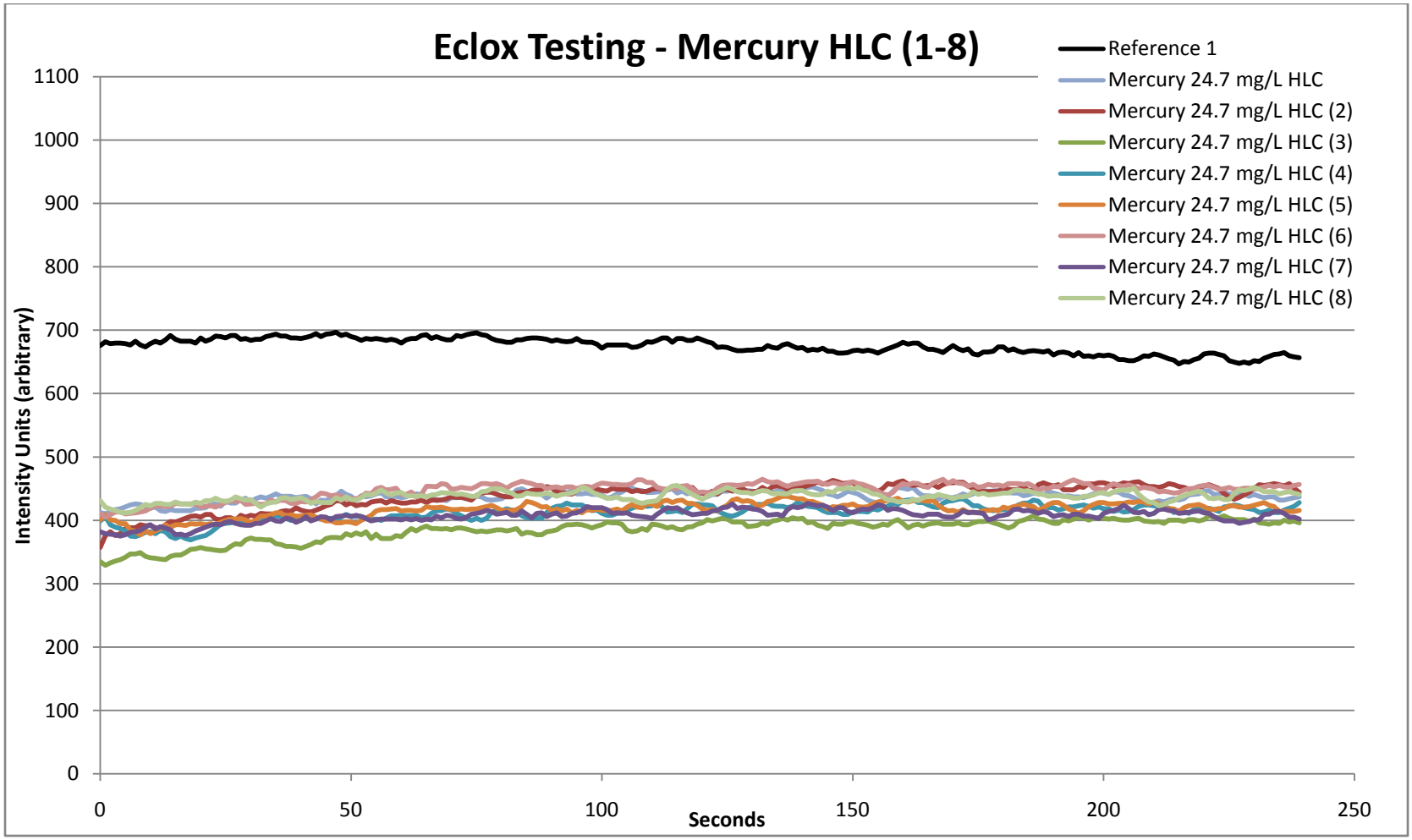

Figure 3-3. Mercury at the Human Lethal Concentration, samples 1-8. Percent inhibition of 39%, 38%, 34%, 35%, 35%, 43%, 40%, 35%, respectively, compared to a reference sample (Millipore water).

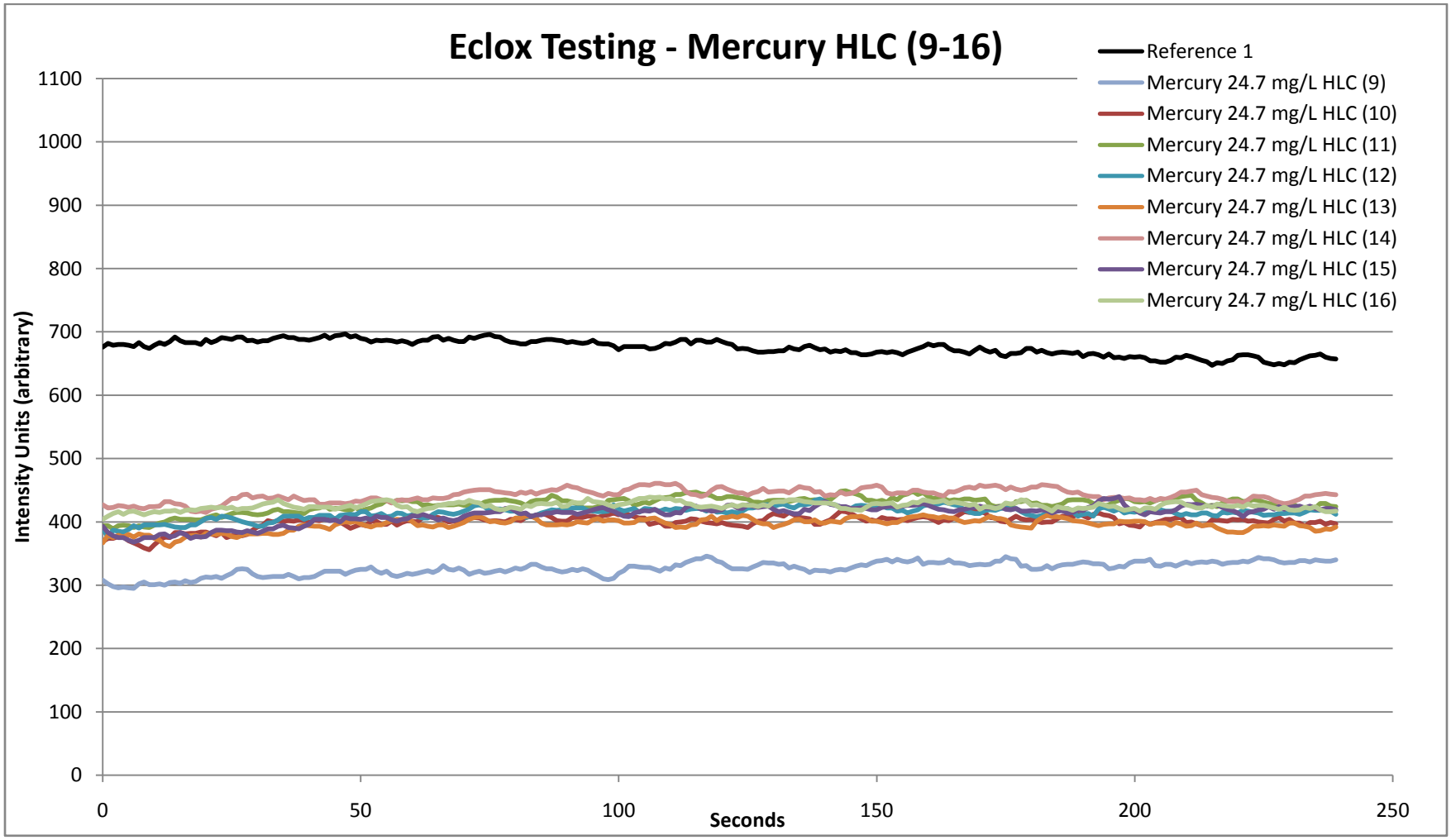

Figure 3-4. Mercury at the Human Lethal Concentration, samples 9-16. Percent inhibition of 52%, 41%, 37%, 39%, 41%, 35%, 39%, 37%, respectively compared to a reference sample (Millipore water).

3.1.4 Eclox Interference Testing

Our testing shows that it is unlikely that the Eclox Chemiluminescence Test will respond to the common disinfectant chloramine, cyanobacterial byproducts (geosmin and MIB) or water hardness that may be present in source or product drinking waters (Table 3-2).

There was a full 100% inhibition response to 10 mg/L of chlorine, but by using the established procedure of page 35 in the Eclox Manual of adding 2 drops of the pre-conditioner solution to a 100 ml sample of chlorinated water, the response due to chlorine was eradicated (3 replicates, with 3%, 0%, and -1% inhibition).

There was a response to humic/fulvic acids at the selected level of 5 mg/L (2.5 mg/L humic acid/2.5 mg/L fulvic acids) for the Eclox Chemiluminescence Test. There was no response to humic/fulvic acids at 0.5 mg/L (0 of 3 replicates responded); however, inhibition at this concentration (21% inhibition) was close to the minimum 25% inhibition level for a response. The MDL for humic/fulvic acids in the Eclox Chemiluminescence Test was 1 mg/L.

Table 3-2: Eclox Interference Chemical Responses

Test Chemicals	Concentration (mg/L)	Response (n=3)	Mean Percent Inhibition (%)
Chlorine	10	Response	100
	10 (with pre-conditioner)	No Response	1
Chloramines	10	No Response	1
Geosmin	0.0001	No Response	5
Methyl-isoborneol (MIB)	0.0001	No Response	-6
Humic / Fulvic Acids (50%/50%)	5 (2.5/2.5)	Response: (3 of 3)	93
	1	Response: MDL (16 of 16)	41
	0.5	No Response	21
Blank – Hard Water	250	No Response	-6

3.2 Pesticide Test Strips

3.2.1 Agri-Screen vs. Hach P/NA Test Strips Results Comparison

One of the research goals of this evaluation was the comparison of the Agri-Screen and Hach P/NA Test strips (Table 3-3). For the OP chemicals (fenamiphos, methyl parathion, and methamidophos) and the carbamate chemicals (aldicarb and oxamyl), the Agri-Screen and Hach P/NA Test strips were tested with at least 3 samples each (Figure 3-4). To maximize the use of resources, non-OP/C chemicals were tested using 3 Agri-Screen samples, 1 Agri-Screen sample with oxidation, and 1 Hach P/NA Test strip. If one of those samples was considered a detect, more replicates were completed.

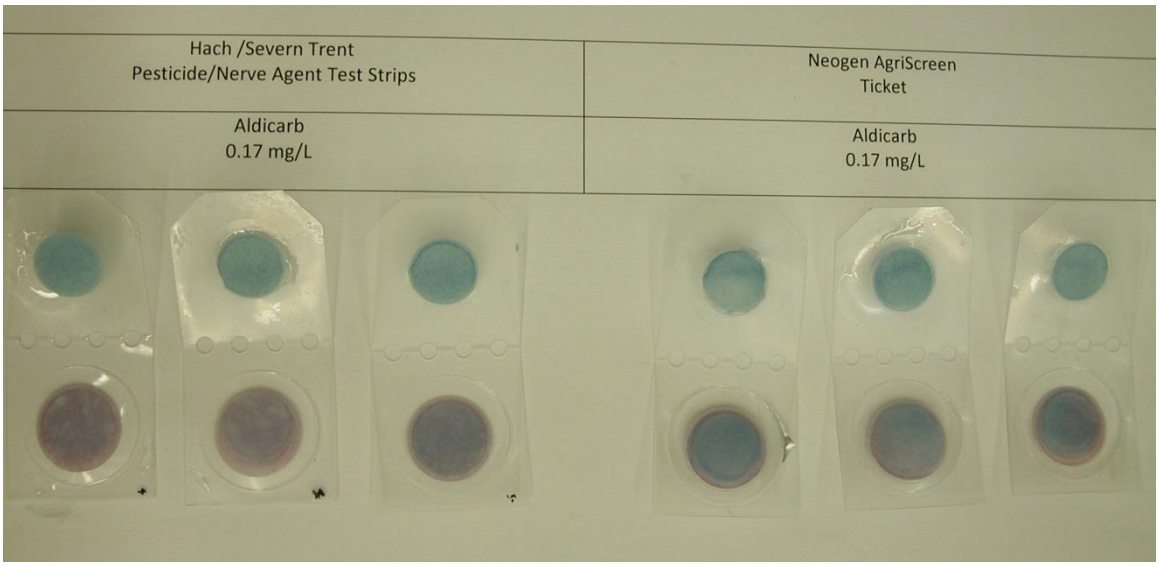

Figure 3-5. Similar negative results from aldicarb (0.17 mg/L) using both the Hach Pesticide/Nerve Agent Test strips and the Agri-Screen Test ticket.

Table 3-3: Agri-Screen and Hach Strip Responses by Chemical and Oxidation Status at the Human Lethal Concentration

Test Chemicals[a]	HLC[b]	*Agri-Screen*		*Hach Pesticide/Nerve Agent Strips*	
		Detected? (n=3)[c]	With oxidation (n=1)	Detected? (n=1)	With oxidation[d]
Acrylonitrile	4.2	ND[e]	ND	ND	---[f]
Aldicarb	0.17	ND	ND	ND (n=3)	---
Ammonia	924	ND	ND	ND	---
Arsenic (sodium arsenite)	4.5	ND	ND	ND	---
Azide (sodium azide)	47	ND	ND	ND	---
Copper (sulfate)	103	Yes (16 of 16)	Yes (1 of 1)	Yes (1 of 1)	---
Cyanide (sodium)	14	ND	Yes (1 of 3)	ND	ND (n=1)
Ethylene glycol	3157	ND	ND	ND	---
Fenamiphos	0.56	ND	ND	ND (n=3)	---
Fluoroacetate (sodium)	3.9	ND	ND	ND	---
Mercury (chloride)	24.7	ND	ND	ND	---
Methamidophos	1.4	ND	ND	ND (n=3)	---
Methyl parathion	33.6	ND	Yes, 2.3 mg/L (16 of 16)	ND (n=3)	Yes, 2.3 mg/L (3 of 3)
Nicotine	16.8	ND	ND	ND	---
Oxamyl	0.63	Yes (15 of 16)	Yes (1 of 1)	Yes (n= 3)	---
Paraquat (dichloride)	4.6	ND	ND	ND	---
Pentachlorophenate (sodium)	71.9	ND	ND	ND	---
Phenol	91.5	ND	ND	ND	---
Strychnine	1.3	ND	ND	ND	---
Thallium (sulfate)	13.5	ND	ND	ND	---
Toluene	840	ND	ND	ND	---

[a] More chemical information available in Appendix D
[b] **HLC** – Human Lethal Concentration (70 kg person, 15 L/day)
[c] **n** – number of samples
[d] oxidation step performed using oxidation materials provided with the Agri-Screen tickets
[e] **ND** – not detected
[f] --- Not tested

Detected

Since the Hach P/NA Test strips do not include an oxidation step, this product does not detect the thio-organophosphate pesticides (e.g., methyl parathion). However, the oxidation materials from the Agri-Screen tickets were applied to the Hach P/NA Test strips for a simple comparison of the strips. Another instance where the Agri-Screen data was not consistent with the Hach P/NA Test strips was with cyanide. However, only 1 of 3 of the Agri-Screen tickets responded at the cyanide HLC. Therefore, based on the data in Table 3-3, the Neogen ® Agri-Screen tickets and the Hach P/NA Test strips respond

very closely to toxic chemical challenges and may in fact be the same test system considering their nearly identical physical appearance.

3.2.2 Abraxis O/PC Screen Test vs. Pesticide Test Strips/Tickets

Another research goal was to compare the response of both the Agri-Screen tickets and the Hach P/NA Test strips to the Abraxis OP/C Screen Test (Table 3-4). The Abraxis OP/C Screen detected 7 chemicals in the MEG-HLC range, with 2 additional chemicals detected below the MEG (9 of 20). The Agri-Screen pesticide test strips detected 3 chemicals in the MEG-HLC range. These pesticide test strips were able to detect 6 chemicals above the HLC. No testing was completed above the HLC for any Abraxis OP/C testing. For the 3 chemicals that these technologies detected, (methyl parathion, oxamyl, and copper) the Abraxis OP/C Screen has a lower detection limit. The Abraxis OP/C Screen is superior to Agri-Screen tickets in both the number of chemicals detected below the HLC (9 vs. 3) and in lower detection limits of commonly detected chemicals. Additionally, the OP/C Screen is superior to the Hach P/NA Test strips as they are currently packaged (9 chemicals detected vs. 2). The Hach P/NA Test strips could detect a third chemical (methyl parathion) using the Agri-Screen oxidation step (Figure 3-6), but oxidation materials are not provided with the Hach P/NA Test strips.

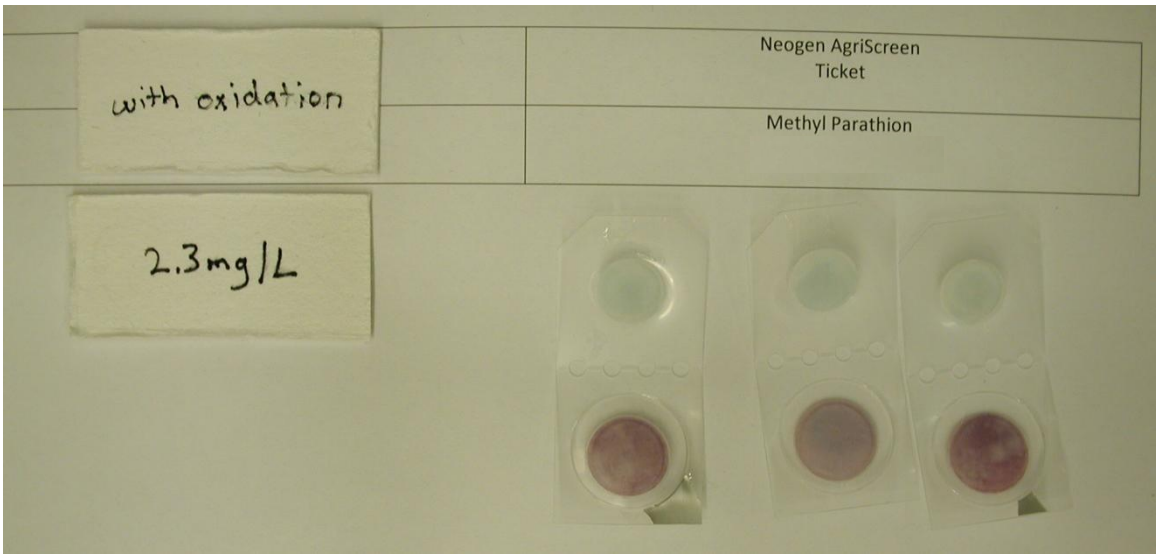

Figure 3-6. Methyl parathion samples 1-3 of Agri-Screen with oxidation

Table 3-4: Comparison of Three Pesticide Detection Technologies

Test Chemicals (mg/L)	MEG[a]	Abraxis OP/C Screen MDL[b]	Agri-Screen MDL	Hach P/NA Test Strips MDL	HLC[c]
OP[d] and Carbamate Pesticides					
Aldicarb	**0.005**	0.02	0.67 – 6.7	0.67 – 6.7	**0.17**
Fenamiphos	**0.004**	0.08	0.56 – 204	0.56 – 204	**0.56**
Methamidophos	**0.002**	1.25	101 – 1008	101 – 1008	**1.4**
Methyl parathion	**0.15**	0.016	2.3[e]	>33.6	**33.6**
Oxamyl	**0.1**	0.04	0.63[f]	0.63	**0.63**
Non-OP and Carbamate Chemicals					
Acrylonitrile	**0.14**	>4.2[g]	>4.2	>4.2	**4.2**
Ammonia	**30**	>924	>924	>924	**924**
Arsenic (sodium arsenite)	**0.02**	>4.5	46	46	**4.5**
Copper (sulfate)	**0.14**	52	103	103	**103**
Cyanide (sodium)	**2**	3.5	>14	>14	**14**
Ethylene glycol	**2.5**	>3157	> 3157	> 3157	**3157**
Fluoroacetate (sodium)	**0.0009**	>3.9	>4211	>4211	**3.9**
Mercury	**0.001**	12.4	>94.1	>94.1	**24.7**
Nicotine	**0.13**	>16.8	16.8 – 168	16.8 – 168	**16.8**
Paraquat (dichloride)	**0.05**	>4.6	>46	>46	**4.6**
Pentachlorophenate (sodium)	**0.14**	50	71.9 – 2154	71.9 – 2154	**71.9**
Phenol	**3**	>91.5	>393.8	>393.8	**91.5**
Strychnine	**0.014**	>1.3	>47.9	>47.9	**1.3**
Thallium (sulfate)	**0.003**	>13.5	>465.5	>465.5	**13.5**
Toluene	**30**	>840	>444	>444	**840**
Azide (sodium)	**0.12**	Not tested	>47	>47	
Legend		Abraxis	Agri-Screen	Hach	
Chemical detected in MEG-HLC range		7	3	2	
Chemical detected below MEG		2	0	0	
Chemical detected above HLC		11 (none tested above HLC)	6	6	
Chemical not detected			12	13	

[a] **MEG** – 7 to 14 day Military Exposure Guidelines (15 L/day), when available, 1 year MEG for copper, fluoroacetate, and strychnine; < 7 day MEG for nicotine; fenamiphos MEG estimated from terbufos (Richards, personal communication), [b] **MDL** – Minimum Detection Limit where n=16 with 0 false negatives, [c] **HLC –** Human Lethal Concentration (70 kg person, 15 L/day) [d] **OP** – Organophosphate, [e] Methyl parathion detected with oxidation only, [f] Oxamyl detected in 15 of 16 tests at the HLC
[g] Abraxis samples were not tested above the HLC

3.2.3 Previous Battelle Screening vs. Definitive Testing

Table 3-5: Agri-Screen Organophosphate and Carbamate Responses				
	Battelle, (2008)		USACEHR	
Test Chemical	Concentration (mg/L)	Response (n=1)	Concentration (mg/L)	Response (n=3)
Aldicarb	0.0005	ND	0.17 (HLC)	ND
	0.005	ND	0.67	ND
	0.05	ND	6.7	Detected (3 of 3)
	0.17 (HLC)	ND		
Fenamiphos	0.0004	ND	0.56 (HLC)	ND
	0.004	ND		
	0.04	ND	203.77	Detected (1 of 1)
	0.56 (HLC)	ND		
Methamidophos	0.002	ND	1.4 (HLC)	ND
	0.02	ND	100.8	ND
	0.2	ND	1008	Detected (1 of 1)
	1.4 (HLC)	ND		
Methyl Parathion	0.015	ND	0.15 (HLC)	ND
	0.15	ND	2.3	Detected (16 of16)
	1.5	ND		
	33.6 (HLC)	Detected	33.6	Detected (3 of 3)
Oxamyl	0.001	ND	0.63 (HLC)	Detected (15 of16)
	0.01	ND		
	0.1	ND		
	0.63 (HLC)	ND		

Battelle (2008) evaluated the sensitivity of several commercial off-the-shelf enzyme test kits, including the Agri-Screen test, to OP and carbamate pesticides (Table 3-5). The Battelle Agri-Screen data provide a reference point for tests conducted as a part of the current effort. In USACEHR testing, aldicarb, fenamiphos, and methamidophos were only detected at the stock concentrations (6.7 mg/L, 203.77 mg/L, and 1008 mg/L, respectively). Methyl parathion was tested using the Agri-Screen test at the geometric mean of 2.3 mg/L between the MEG (0.15 mg/L) and the HLC (33.6 mg/L), after 3 of 3 replicates at the HLC were detected. Previous testing with Battelle did not detect methyl parathion at 1.5 mg/L, however in these tests it was detected 16 of 16 times at 2.3 mg/L (Figure 3-7), but only when the oxidation step was used. The Hach P/NA Test strips could not detect methyl parathion, since these strips do not come with the oxidation step, which is necessary to detect thio OPs. However, the Hach P/NA Test strips did detect methyl parathion when the Agri-Screen oxidation step was added to the procedure (Figure 3-8).

Oxamyl was detected 6 of 6 times during the initial testing phases at the HLC (0.63 mg/L). The final set of 10 replicates, proved to have 1 non-detect, yielding a 15 of 16 detection result. However, somewhat faint blue stains on the white pads were noted on some samples. The Battelle screening indicated no response for oxamyl at the HLC. Battelle only tested one replicate, however, because definitive testing was not part of their study.

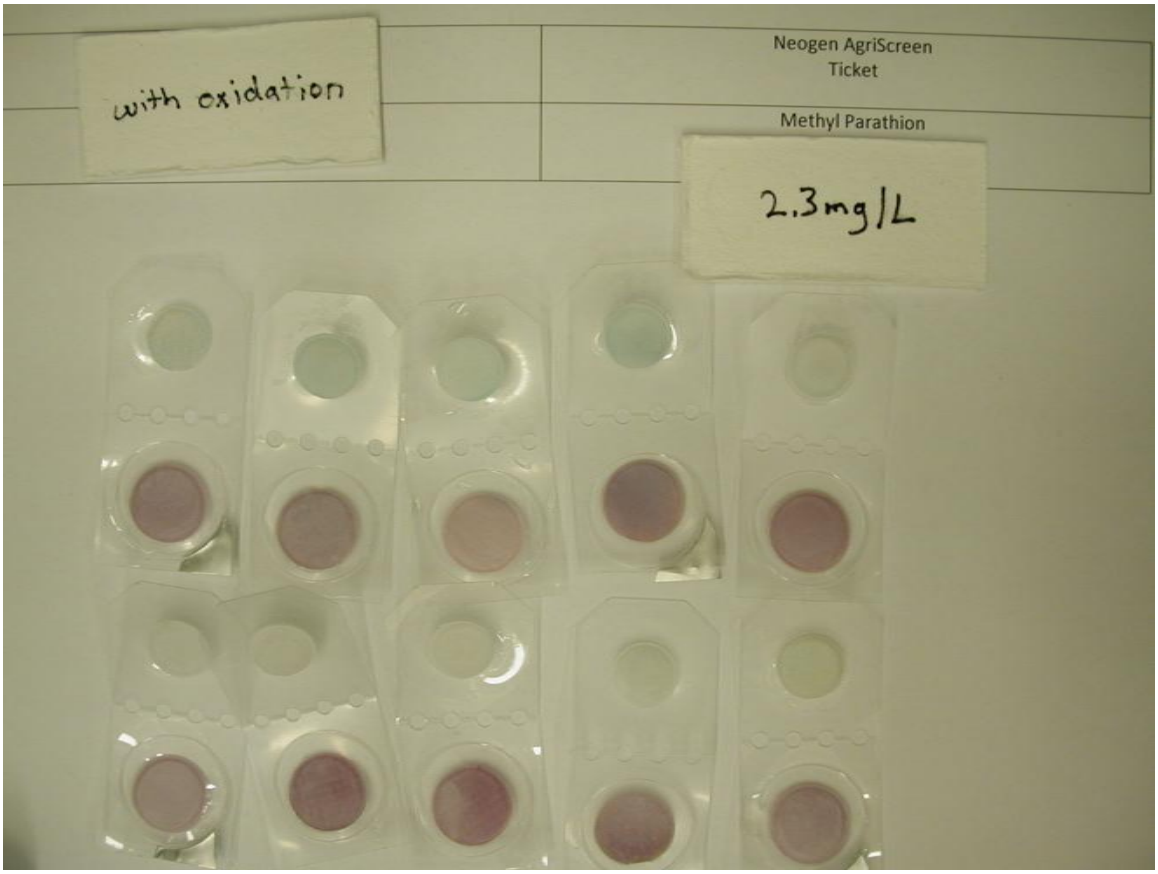

Fig. 3-7. Methyl Parathion at 2.3 mg/L Sample numbers 7-16 using the Agri-Screen Test Ticket with the oxidation step.

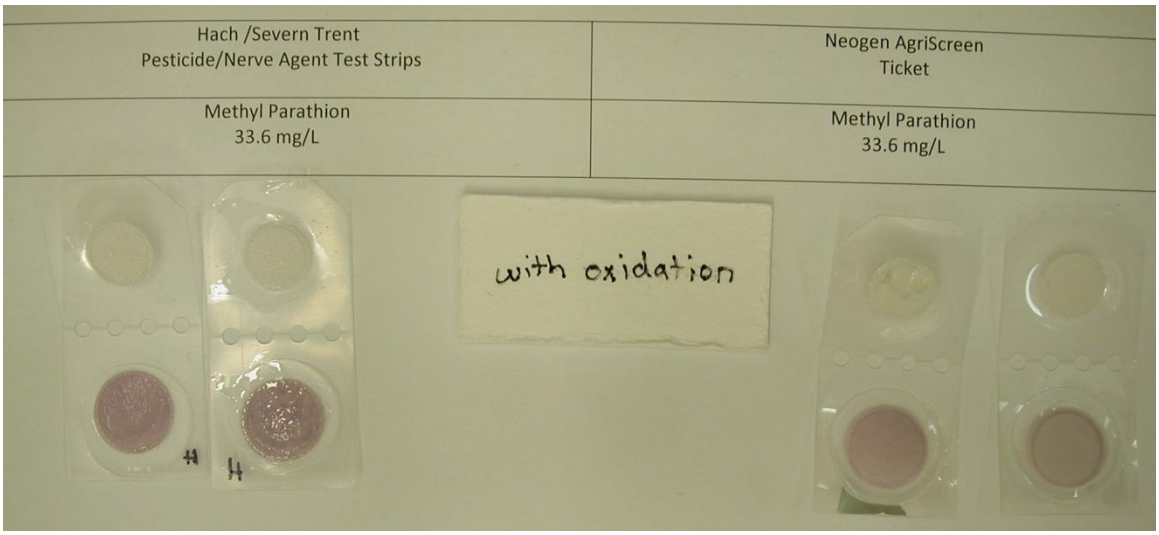

Figure 3-8. Methyl parathion at 2.3 mg/L, samples 2 and 3, for both the Hach Pesticide / Nerve Agent Test strips and the Agri-Screen tickets, each using the Agri-Screen oxidation step.

3.2.4 Pesticide Strip Interference Testing – Pesticide Strips

There was no response to the interferences at the tested levels for either the Agri-Screen or the Hach P/NA Test strips (Table 3-6). Our testing shows that it is unlikely that either pesticide strip will respond to common disinfectants (chlorine and chloramine), cyanobacterial byproducts (geosmin and MIB) or water quality parameters (humic/fulvic acids or water hardness) that may be present in source or product drinking waters.

Table 3-6: Agri-Screen and Hach P/NA Test Strip Interference Chemical Responses

Test Chemicals	Concentration[a]	Response[b]
Chlorine	10	No Response
Chloramines	10	No Response
Geosmin	0.0001	No Response
Methyl-isoborneol (MIB)	0.0001	No Response
Humic / Fulvic Acids (50%/50%)	5 (2.5/2.5)	No Response
Blank – Hard Water	250	No Response

[a] All concentrations reported as milligrams/liter
[b] Agri-Screen (n=3) and Hach P/NA Test strips (n=1)

3.3 Conclusions

The Eclox Chemiluminescence Test is a robust test with sturdy packaging and materials. It is a very simple procedure, has only a few reagents (3), and results are ready within 4 minutes. In addition, the reagents are stable for 1 year in refrigeration either in packaged or diluted form, 4 months at +40°C in packaged form, or 72 hours at +40°C in diluted form. Both the Agri-Screen and the Hach P/NA Test strip methods are incredibly simple

to use. The Hach P/NA Test strips require no reagents, and results are ready within 4 minutes. The Neogen ® Agri-Screen test tickets requires only 1 reagent and results are ready within 7 minutes. The Agri-Screen is stable at room temperature for 1 year.

However, the Eclox and Hach P/NA Test strip combination only responds to 2 of 21 chemicals in the MEG-HLC range, and only 4 of 21 below the HLC. If the Agri-Screen tickets with the included oxidation step were substituted for the Hach P/NA Test strip, then the total number of chemicals detected below the HLC would be 5 of 21.

Acknowledgments

We thank Alan Rosencrance and Bill Dennis for analytical chemistry support, Linda Brennan for chlorine and chloramine analysis, and Mark Widder, Tom Gargan, and Tommy Shedd for their overall support and efforts with the ESB system. We also thank Keith McLeroy at TEEX for the Eclox training and data sharing he provided. Elgin Perry provided the statistical analysis to determine the false positive rate for the Eclox test. This work was funded by U.S. Army Medical Research and Materiel Command, Task Area 3.D., "Environmental Sentinel Biomonitor (ESB) System for Rapid Determination of Toxic Hazards in Water." The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

References

ASTM 6592-01. 2001. Section 11 Water and Environmental Technology. Standard Test Method for Portable Chemiluminescent Water Quality Determination. West Conshohocken, PA. 1088-1095

Buehler, S. 2008. W81XWH-05-D-0001, Task Order 0015. Comparative Analysis of Enzymatic Test Kits for Detecting Pesticides in Drinking Water Final Report. Battelle, Columbus, OH. June

Eclox™ Water Test Kit User Manual. May 2006, Edition 2. Hach Company, Loveland, CO. www.hach.com

Henderson, B. Water Sector Security: A State Perspective. http://74.125.113.132/search?q=cache:aP9BNeHbwRkJ:www.nawc.org/events/past-events/2005-DWS/tuesday/Henderson-Water%2520Sector%2520Security-2005%2520NAWC%2520Symposium.pdf+eclox+alabama&cd=8&hl=en&ct=clnk&gl=us&lr=lang_en

Hogan, J., B. Harper, L. Neuendorff, and J. Seerup. 2007. Detailed Test Plan for Handheld Assay (HHA) Testing for the Joint Chemical, Biological, and Radiological Agent Water Monitor (JCBRAWM) Increment 1, Production Qualification Test (PQT). Test Project No. 2007-DT-DPG-JCBRA-DA182, WDTC Document No. WDTC-OP-07-033. West Desert Test Center, U. S. Army Dugway Proving Ground, Dugway, UT 84022-5000, 82 pages.

Jane's Nuclear, Biological and Chemical Defense. June 18, 2002. Eclox™ Military Water Test Kits (Biological and Chemical) (WTK (B&C)) (United Kingdom), Detection (Sensor Systems) – Chemical.

Kooistra, S., J. Walther and L. Wurster. 2007. Environmental Sentinel Biomonitor (ESB) System Technology Assessment. ECBC-TR-517, Edgewood Chemical Biological Center, U.S. Army Research, Development, and Engineering Center, Aberdeen Proving Ground, MD.

Lauer, B. 2005. Water quality in the distribution system. Chapter 32 Evaluation of the DeltaTox and Eclox systems for water toxicity screening, Hughley et al. pg. 655-688.

Severn Trent Services, Inc. Eclox Rural Water Association Helps Assure Water Security Statewide with Rapid Response Water Test Kit. 1 June 2006. http://www.environmental-expert.com/resultEachArticle.aspx?cid=8563&codi=21381&level=0

States,S.; Scheuring,M.; Kuchta,J.; Newberry,J.; Casson,L.. Utility-based analytical methods to ensure public water supply security. 2003. Journal of the American Water Works Association, 95 (4): 103-115.

Toxicology Excellence for Risk Assessment (TERA). 2006. Derivation of Human Lethal Doses. USACEHR Technical Report 0802, U.S. Army Center for Environmental Health Research, Fort Detrick, MD. ADA494706.

Trader, D.E., Gargan, II, T.P, and W. H. van der Schalie. 2009. An Evaluation of the Abraxis Organophosphate/Carbamate Screen Test. USACEHR-TR-0901. Defense Technical Information Center Accession Number: ADB351100. U.S. Army Center for Environmental Health Research, Fort Detrick, MD.

U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), Version 1.3—Updated May 2003 with January 2004 Addendum. Chemical Exposure Guidelines for Deployed Military Personnel. Technical Guide (TG) 230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD. (http://chppm-www.apgea.army.mil/documents/TG/TECHGUID/TG230.pdf)

van der Schalie, W.H., R.R. James, and T.P. Gargan, II. 2006. Selection of a Battery of Rapid Toxicity Sensors for Drinking Water Evaluation. *Biosensors and Bioelectronics* 22:18-27.

Widder, M.W., L.M. Brennan, D.E. Trader, and W.H. van der Schalie. 2007. Rapid Toxicity Sensor Responses to Selected Chemicals in Water. USACEHR Technical Report 0701, ADB327259, U.S. Army Center for Environmental Health Research, Fort Detrick, MD.

List of Abbreviations and Acronyms

%	Percent
µL	microliter
AChE	acetylcholinesterase
ASTM	American Society of Testing and Materials
°C	degree Celsius
ECIS	Electric Cell-substrate Impedance Sensing
ESB	Environmental Sentinel Biomonitor
HLC	human lethal concentration
IPT	Integrated Product Team
kg	kilogram
JCBRAWM	Joint Chemical Biological Radiological Agent Water Monitor
L	liter
M	Molar
MDL	minimum detection limit
MEG	Military Exposure Guidelines
mg	milligram
min	minute
MIB	methyl iso-borneol
mM	millimolar
ND	no detection
NT	not tested
OP	organophosphate

OP/C	organophosphate and carbamate
P/NA	Pesticide / Nerve Agent
PCP	pentachlorophenate
TEEX	Texas Engineering Extension Services
TICs	Toxic Industrial Chemicals
USACEHR	U.S. Army Center for Environmental Health Research
USACHPPM	U.S. Center for Health Promotion and Preventative Medicine

Appendix A
Neogen® Agri-Screen and Hach Pesticide Strip Procedure

Summarized Test Procedure for the Neogen ® Agri-Screen Test Ticket and Hach Pesticide/Nerve Agent Strip

A complete method and picture procedure is available at the following websites: http://www.neogen.com/FoodSafety/pdf/ProdInfo/Page_102.pdf and http://www.biolabgroup.com/AUS/LAB/pdf/MicroBiol/AddRapidTest/Neogen/Ticket%20Lit.pdf

Note: The Hach strip does not have an activator ampoule, so omit steps 2 and 3 if using the Hach Pesticide/Nerve Agent Test strips

1. Place 20 ml of water sample into 50 ml glass beaker
2. Place activator (oxidizing chemical – bromine) ampoule into beaker and break using glass stir rod.
3. Wait 3 minutes.
4. Remove ticket from packet and peel back the foil on the white pad side only. Place this exposed half of the ticket into the sample and hold it there for 1 minute
5. Remove the ticket from the sample, and peel the rest of the foil off the ticket. Bend the ticket in half, touching the white and blue pads to each other and pinch for 3 minutes (you can also use the copper clip or a paper binder clip).
6. Open the ticket and read immediately. A white pad and blue pad means the sample tested positive for a pesticide, and two blue/purple pads mean the sample tested negative for a pesticide.

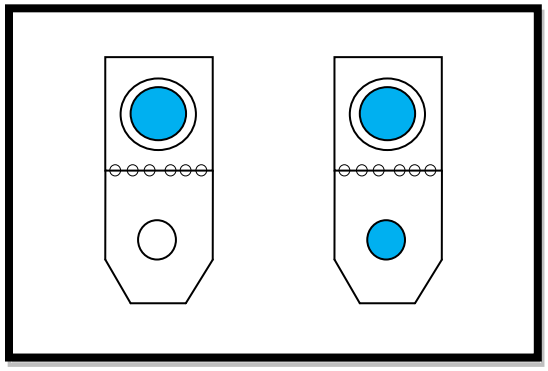

Illustration of a positive and negative result of the strip/ticket technology. A white disk and blue disk are positive (left, detect) and 2 blue disks are negative (right, non-detect).

Appendix B
Eclox Chemiluminescence Procedure

Summarized Procedure for the Eclox Chemiluminescence Test

A complete procedure and manual is available at the following website:
http://www.hach.com/fmmimghach?/CODE%3A2886888_2ED9467%7C1

1. Prepare the reagents according to Section 4 of the Eclox manual.
2. Turn on the Eclox and use pre-conditioner if greater than 0.4 mg/L chlorine is present. Several internal tests will check the luminometer and internal software.

Create a Reference Sample

3. Select Measure, then press ENTER, select Measure Reference, then press ENTER.
4. Open the luminometer lid and remove any remaining sample, then shut the lid.
5. Press PROCEED, then PROCEED again after the luminometer performs internal tests and cell zeroing.
6. Place a cuvette into the black cuvette holder.
7. Place 1000 µl (or 1 ml) of the reference sample (Millipore water) into the cuvette using a calibrated automatic pipettor and 1000 µl pipette tip.
8. Place 100 µl of Reagent 1 into the cuvette with sample.
9. Place 100 µl of Reagent 2 into the cuvette with sample.
10. Place 100 µl of Reagent 3 into the cuvette with sample.
11. Pulse vortex the sample or tap the cuvette on the work surface to mix the sample
12. Lift the lid of the luminometer and place the cuvette into the cell.
13. Close the lid of the luminometer and press PROCEED. The luminometer will run for 4 minutes and display DONE.

Measure a Sample

1. Select Measure, then press ENTER, select Measure Sample, then press ENTER.
2. Open the luminometer lid, remove any remaining sample, and then shut the lid.
3. Press PROCEED, then PROCEED again after the luminometer performs internal tests and cell zeroing.
4. Place a cuvette into the black cuvette holder.
5. Place 1000 µl (or 1 ml) of water sample into the cuvette using a calibrated automatic pipettor and 1000 µl pipette tip.
6. Place 100 µl of Reagent 1 into the cuvette with sample.
7. Place 100 µl of Reagent 2 into the cuvette with sample.
8. Place 100 µl of Reagent 3 into the cuvette with sample.
9. Pulse vortex the sample or tap the cuvette on the work surface to mix the sample
10. Lift the lid of the luminometer and place the cuvette into the cell.
11. Close the lid of the luminometer and press PROCEED. The luminometer will run for 4 minutes and display DONE. A graph will display the intensity units over time for both the reference sample and the current sample. A % inhibition will also be displayed.

Appendix C
Negative Control Statistics and Threshold Determination

Report on Negative Control results and expected false positive rates for the ECLOX assay.

The reaction produces maximum light output where no contamination is present, as represented by the Reference Sample. This is to be compared with light output from a Treatment Sample which may or may not contain contamination by computing percent inhibition. For each sample, total light output is measured as the area under the curve represented by a sequence of luminosity readings taken at 1-second intervals over a period of four minutes for a total of 240 readings. Because the readings are equally spaced, a simple approximation of the total light can be obtained using the rectangle rule for numerical quadrature by simply summing the light readings for each sequence. These calculations would be as follows:

$$TLR = \sum_{i=1}^{240} LR_i$$

where:

TLR = total light for the reference sample
LR_i = reference light or luminosity reading for each second i = 1,2, . . . 240.

$$TLT = \sum_{i=1}^{240} LT_i$$

TLT = total light for the treatment sample
LT_i = treatment light or luminosity reading for each second i = 1,2, . . . 240.

Given total light for the reference and treatment samples, percent inhibition is computed as follows:

$$PI = \left[1 - \frac{TLT}{TLR}\right] \times 100\%$$

where:

PI = percent inhibition and other terms are given above.

Distributional properties of initial 30 blank samples for percent inhibition using the ECLOX assay:

Below is what the distribution and false positive rate estimates would look like if just the initial 30 samples were used.

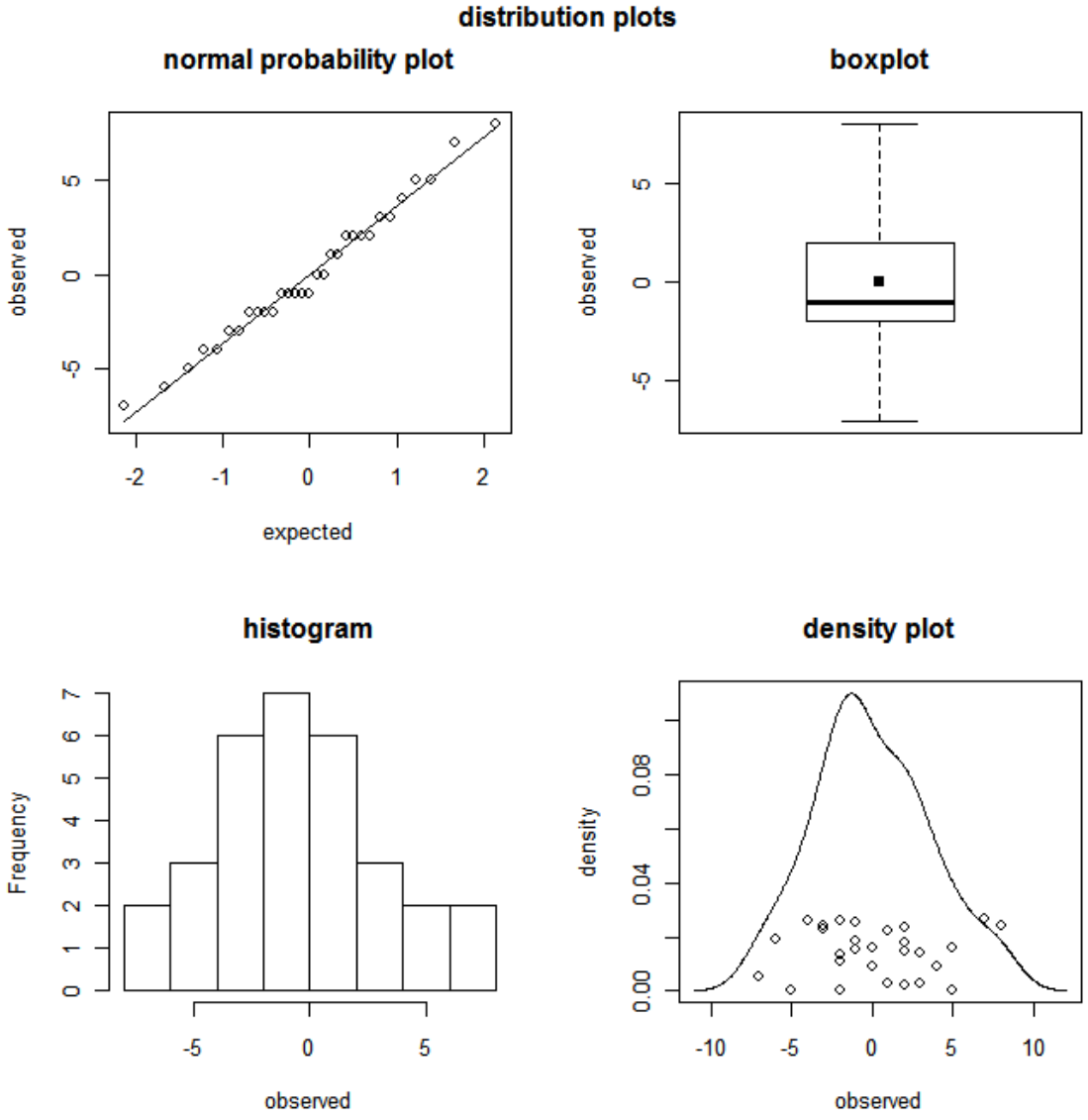

Figure 1. Graphical assessment of distributional properties of 30 negative control samples of percent inhibition using the ECLOX assay.

The distribution of percent inhibition (figure 1) looks to conform to very well to the normal distribution. This leads us to believe that computing critical points for false positives using the normal distribution should work well.

Table 1. Summary statistics for the distribution of 30 negative control samples of percent inhibition using the ECLOX assay.

statistic	estimate
sample size	30
mean	0.0000000
standard dev	3.6952906
variance	13.6551724
skewness	0.2338494
excess kurtosis	-0.6231303
minimum	-7.0000000
q25	-2.0000000
median	-1.0000000
q75	2.0000000
maximum	8.0000000

The 30 observations of percent inhibition have a range from -7 to 8 and a standard deviation of 3.7 (Table 1.). From these estimates, we calculated Critical Points for the percent inhibition distribution that should insure a given false positive rate. The formula for the calculation is

Inhibition Critical Point = abs(Gaussian Critical Point(p) x standard deviation)

where:
standard deviation = the standard deviation of percent inhibition (Table 1.)
Gaussian Critical Point(p) = the pth quantile of the Gaussian or Normal(0,1) distribution
p = probability of a false positive

Using these methods and these data, we obtain the results in Table 2.

Table 2. Probability levels and associated critical points for assessing the false positive rates of the ECLOX assay based on 30 negative control samples.

probability level	Gaussian Critical Point	Inhibition Critical Point
0.05	-1.6449	6.1
0.01	-2.3263	8.6
0.005	-2.5758	9.5
0.001	-3.0902	11.4
0.0005	-3.2905	12.2
0.0001	-3.7190	13.7

From this we would conclude that using a percent inhibition critical point of 15% would insure a false positive rate of only 1 in 10,000.

Distributional properties of ECLOX negative control blanks based on all possible pairings of blank samples.

Here we apply the same methods described above to a larger sample created by computing percent inhibition for all possible pairings (31 x 30 /2 = 465) of the negative control samples.

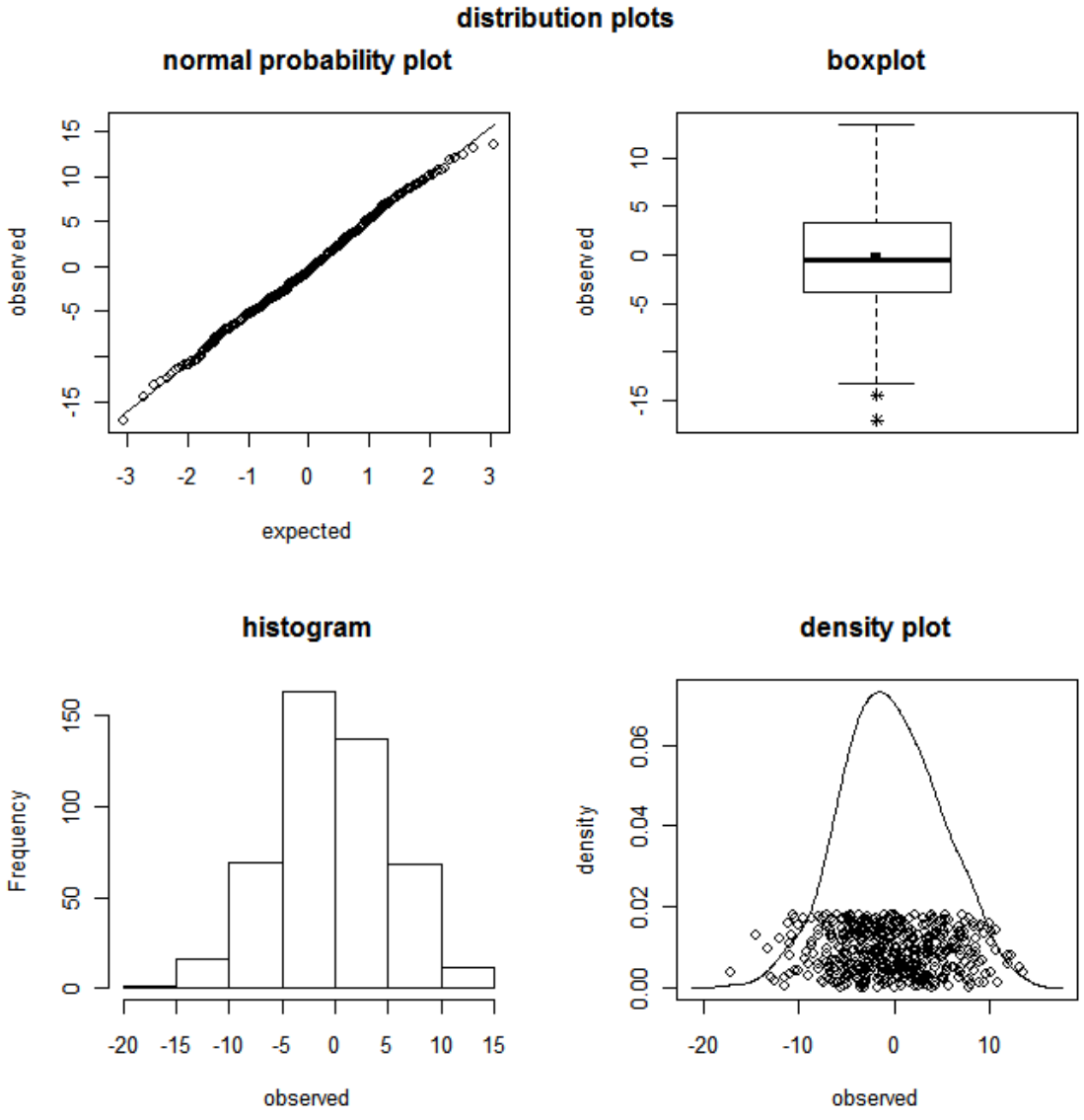

Figure 2. Graphical assessment of distributional properties of 465 negative control samples of percent inhibition using the ECLOX assay.

Again the data appear to conform well to the expectations from a normal distribution (figure 2.).

Table 3. Summary statistics for the distribution of 465 negative control samples of percent inhibition using the ECLOX assay.

statistic	estimate
sample size	465.00000000
mean	-0.32612803
standard dev	5.24631038
variance	27.52377259
skewness	0.01433429
excess kurtosis	-0.19945874
minimum	-17.08677533
q25	-3.77690725
median	-0.52900565
q75	3.28831980
maximum	13.48452738

The summary statistics (Table 3.) give us the impression that the distribution is broader than that of the 30 initial samples. The range extends from -17 to 13 and the standard deviation is 5.25. I believe this is because the total luminosity of sample 1 (the reference) is 102901 which is very near to the mean luminosity of all 31 samples (102906) in a range of 94301 to 110414. Because it is near to the mean, it minimizes variance when compared to other samples. I believe this broader distribution is more realistic.

Table 4. Probability levels and associated critical points for assessing the false positive rates of the ECLOX assay based on 465 negative control samples.

probability level	Gaussian Critical Point	Inhibition Critical Point
0.05	-1.6449	8.6
0.01	-2.3263	12.2
0.005	-2.5758	13.5
0.001	-3.0902	16.2
0.0005	-3.2905	17.3
0.0001	-3.7190	19.5

From this we would conclude that the percent inhibition critical point should be 20% to insure a false positive rate of only 1 in 10,000.

Appendix D
Chemicals Evaluated

Appendix D: Chemicals Evaluated

Compound [measured analyte]	Chemical Abstracts Service Number[a]	Storage Requirements	Analytical Method	Source	Stability in Deionized Water	Purity %
Acrylonitrile [acrylonitrile]	107-13-1	4º C / dark	HPLC	Chem Service West Chester, PA	<3 hrs - open container;14 days - no head-space vial	99.5
Aldicarb [aldicarb]	116-06-3	4º C / dark	HPLC	Chem Service West Chester, PA	>14 days	99
Ammonium chloride [total ammonia]	12125-02-9	4º C / dark	colorimetric	Sigma-Aldrich St. Louis, MO	>14 days	99.99
Sodium arsenite [As]	7784-46-5	4º C / dark	ICP-MS	Chem Service West Chester, PA	>14 days	98
Sodium azide [azude]	26628-22-8	4º C / dark	Ion Chromatograph	Sigma-Aldrich	>14 days	99.5
Chloramine [monochloramine]	10599-90-3	4º C / dark	amperometric titration	Sigma-Aldrich	24 hrs	NA
Sodium hypochlorite [chlorine residual]	76881-52-9	4º C / dark	amperometric titration	Riedel-de Haën Fine Chemicals Seelze Germany	>14 days	NA
Copper sulfate [Cu]	7758-99-8	4º C / dark	ICP-MS	Sigma-Aldrich	>14 days	99.95
Sodium cyanide [cyanide]	143-33-9	4º C / dark	ion probe	Sigma-Aldrich	>14 days	99.98
Ethylene glycol [ethylene glycol]	107-21-1	4º C / dark	Nominal	Sigma-Aldrich	not measured[b]	99.8
Fenamiphos [fenamiphos]	22224-92-6	room temp / dark	Nominal	Chem Service	>14 days	98.5
Sodium fluoroacetate [fluoroacetate]	62-74-8	4º C / dark	HPLC	Sigma-Aldrich	> 14 days	>90
Geosmin	19700-21-1	4º C / dark	Nominal	Sigma-Aldrich	not measured[b]	98
Humic/fulvic acid mixture (1:1 by weight)	NA	4º C / dark	Nominal	International Humic Substances Society, St. Paul, MN	not measured[b]	NA
Mercuric chloride [Hg]	7487-94-7	room temp / dark	ICP-MS	Sigma-Aldrich	>14 days	99.5
Methamidophos [methamidophos]	10265-92-6	4º C / dark	Nominal	Chem Service West Chester, PA	>14 days	98.8
Methyl parathion [methyl parathion]	298-00-0	4º C / dark	HPLC	Chem Service West Chester, PA	>14 days	99.3
2-methylisoborneol (MIB)	2371-42-8	4º C / dark	Nominal	Sigma-Aldrich	not measured [b]	98
Nicotine [nicotine]	54-11-5	4º C / dark	HPLC	Chem Service West Chester, PA	>14 days	99.4
Oxamyl [oxamyl]	23135-22-0	4º C / dark	HPLC	Chem Service West Chester, PA	> 14 days	99
Paraquat dichloride [paraquat]	1910-42-5	4º C / dark	HPLC	Chem Service	>14 days	99
Sodium pentachlorophenate [pentachlorophenate]	131-52-2	4º C / dark	HPLC	Mallinckrodt Baker Phillipsburg, NJ	>14 days	99
Phenol [phenol]	108-95-2	4º C / dark	HPLC	Sigma-Aldrich	>14 days	99.5
Strychnine [strychnine]	57-24-9	4º C / dark	HPLC	Sigma-Aldrich	> 14 days	98
Thallium sulfate [Tl]	7446-18-6	4º C / dark	ICP-MS	Sigma-Aldrich	> 14 days	99.995
Toluene [toluene]	108-88-3	4º C / dark	HP6890 GC and HP-7694 HS	Sigma-Aldrich	14 days; no-head space vial	99.8

GC = gas chromatography ····· LC-MS = liquid chromatography – mass spectrophotometry ····· ICP-MS = inductively coupled plasma-mass spectrophotometry ·····
HPLC = high performance liquid chromatography ····· HP6890 GC and HP-7694 HS = gas chromatography & head-space sampling ····· NA = Not available
[a] Number for compound [b] Tested within 24 hrs of preparation